COMPARATIVE EFFECTS OF Cu, CuO, ZnO, AND TiO_2 NANOPARTICLES ON THE ANTIOXIDANT ACTIVITY OF *ARTHROSPIRA PLATENSIS* EXTRACTS

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https://doi.org/10.37904/nanocon.2025.5220

Abstract

The antioxidant activity assessed using the ABTS assay can serve as an indirect marker of moderate oxidative stress, specific to adaptive physiological responses. This study aimed to evaluate the influence of copper nanoparticles (CuNPs), copper oxide nanoparticles (CuONPs), zinc oxide nanoparticles (ZnONPs), and titanium dioxide nanoparticles (TiO_2NPs) on the antioxidant activity of the cyanobacterium *Arthrospira platensis* (spirulina), using the ABTS method. The cyanobacterium was cultivated in mineral medium supplemented with two concentration ranges of nanoparticle: low (0.01–0.5 mg/L) and high (1.0–20 mg/L). Antioxidant activity was measured in both aqueous and ethanolic extracts of the resulting biomass. The results revealed that aqueous extracts exhibited a significant increase in antioxidant activity (77.6–95.3% ABTS inhibition) at higher concentrations of CuNPs, CuONPs, and ZnONPs, following a clear concentration-dependent trend. In ethanolic extracts, the response was more variable: CuNPs induced a moderate increase in antioxidant activity, while CuONPs caused a decrease. TiO_2NPs showed relatively stable antioxidant values across all tested concentrations, with minimal variation between solvents. The study demonstrates that metal-based nanoparticles modulate the antioxidant mechanisms of *Arthrospira platensis*, triggering adaptive responses to oxidative stress, with effects depending on the nanoparticle type, dose, and type of extract. Extracts obtained from exposed biomass may be considered valuable functional products with biotechnological potential.

Keywords: *Arthrospira platensis*, nanoparticles, hydric extract, ethanolic extract, antioxidant activity

1. INTRODUCTION

Microalgae and cyanobacteria represent valuable sources of natural antioxidants [1,2], and their derived products have found applications in the food, pharmaceutical, and cosmetic industries [2,3,4,5]. Extracts obtained from microalgal and cyanobacterial biomass, whose antioxidant activity was assessed by the ABTS assay, have also been tested for antiproliferative, cytotoxic, and antibacterial properties [3,4]. Among them, the cyanobacterium *Arthrospira platensis* (spirulina) stands out for its well-documented antioxidant potential, demonstrated both *in vitro* and *in vivo* [3,5,6]. This species synthesizes a wide spectrum of antioxidant compounds, including phycocyanin, sulfated polysaccharides, carotenoids, phenolic compounds, and antioxidant enzymes [6]. These compounds contribute to neutralizing reactive oxygen species and protect cells against oxidative stress, thereby supporting cellular defense pathways that may participate in adaptive responses [7]. As bioactive ingredients, natural antioxidant complexes offer a superior alternative to synthetic antioxidants [8,9]. It is well established that cultivation conditions strongly influence the antioxidant activity of microalgae and cyanobacteria at both the biomass levels and bioactive extract [10,11]. Moreover, the presence of metals or metallic nanoparticles in the culture medium can significantly modulate these antioxidant properties, either stimulating or inhibiting the synthesis of antioxidant compounds [1,12]. Essential trace elements, such as copper and zinc, are critical for processes including photosynthesis, cellular respiration, and antioxidant protection while metallic nanoparticles, such as CuNPs, CuONPs, ZnONPs, and TiO_2NPs, exhibit photocatalytic properties that can affect growth and secondary metabolism [13]. In *Arthrospira platensis* the antioxidant activity of biomass extracts depends not only on the species intrinsic defense mechanisms but also on the solvent used from extraction [14]. In this context, extracts obtained from biomass exposed to nanoparticles can be considered high-value functional products with potential for biotechnological applications. The present study evaluates the impact of CuNPs, CuONPs, ZnONPs, and TiO_2NPs on the antioxidant activity of extracts derived from *Arthrospira platensis* biomass.

2. EXPERIMENTAL DESIGN

The experiments were conducted using the *Arthrospira platensis* CNMN-CB-02 strain, obtained from the National Collection of Nonpathogenic Microorganisms at the Institute of Microbiology and Biotechnology, Technical University of Moldova. Cultivation was performed in 250 mL Erlenmeyer flasks, with a working volume of 100 mL, using a mineral medium with the following composition: $NaNO_3$ – 2.5 g/L; $NaHCO_3$ – 8.0 g/L; NaCl – 1.0 g/L; K_2SO_4 – 1.0 g/L; Na_2HPO_4 – 0.2 g/L; $MgSO_4 \cdot 7H_2O$ – 0.2 g/L; H_3BO_3 – 0.00286 g/L; $MnCl_2 \cdot 4H_2O$ – 0.00181 g/L; $CuSO_4 \cdot 5H_2O$ – 0.00008 g/L; MoO_3 – 0.000015 g/L. The medium was supplemented with 1 mL/L FeEDTA solution. The cultivation conditions were as follows: 28 ± 2°C, pH 8–10, continuous illumination (37–55 µmol photons/$m^2 \cdot s$), and periodic agitation. The cultivation period was 6 days. To assess the effects of nanoparticles, the medium was supplemented with copper nanoparticles (CuNPs, 25 nm, Sigma-Aldrich), copper oxide nanoparticles (CuONPs, 50 nm, Sigma-Aldrich), zinc oxide nanoparticles (ZnONPs, <100 nm, Sigma-Aldrich), and titanium dioxide nanoparticles (TiO_2NPs, 20 nm, Sigma-Aldrich) at the established concentrations.

The antioxidant activity (AA) of the biomass harvested at the end of cultivation was determined using both aqueous and ethanolic extracts. The biomass, separated from the medium and demineralized, was standardized to 10 mg/mL in distilled water and subjected to repeated freeze-thaw cycles to release intracellular compounds. The supernatant obtained after centrifugation was used as the aqueous extract. To obtain the ethanolic (96%) extract, the biomass recovered after centrifugation was resuspended in 1 mL of ethanol, agitated for 120 minutes at room temperature, and then centrifuged. The supernatant was collected as the ethanolic extract. Antioxidant activity was determined using the ABTS method [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]. The reaction mixture consisted of 0.3 mL of extract and 2.7 mL of ABTS solution (7 mM ABTS activated with 2.45 mM potassium persulfate, with an absorbance of 0.700 ± 0.02 at 734 nm). The reaction time was 6 minutes. Antioxidant activity was expressed as the percentage of ABTS inhibition [15].

All analyses were performed in triplicate. Significant differences between experimental variants were analyzed using the Student's t-test (two-tailed), with the significance threshold set at $p < 0.05$. Correlations between the applied nanoparticle concentrations and antioxidant activity values were evaluated using the Pearson correlation coefficient.

3. RESULTS AND DISSCUSION

The antioxidant activity of aqueous extracts obtained from *Arthrospira platensis* biomass treated with nanoparticles showed significant variations, depending on the concentration and type of nanoparticles applied (**Figure 1**).

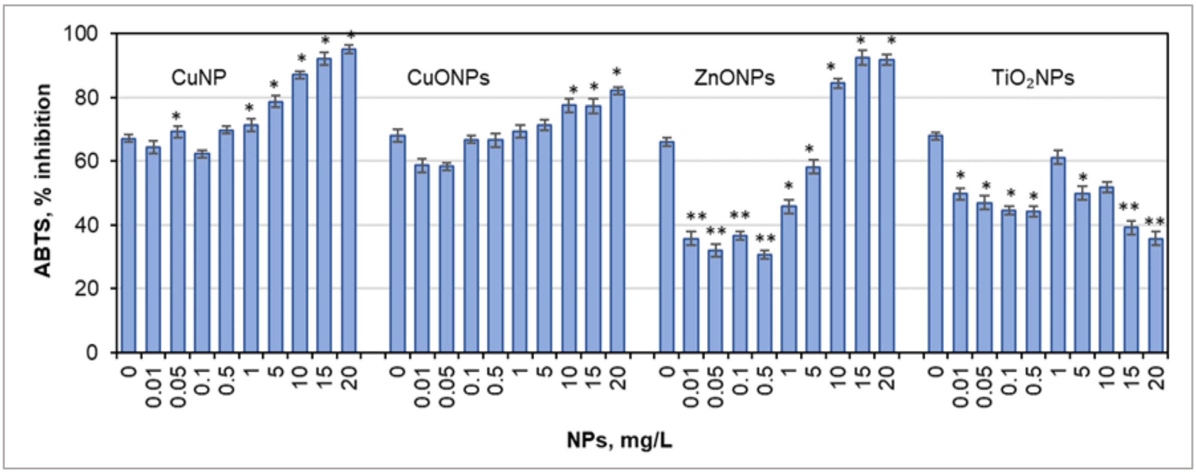

Figure 1 Antioxidant activity (% ABTS inhibition) of aqueous extracts obtained from the biomass of *Arthrospira platensis* exposed to CuNPs, CuONPs, TiO_2NPs, and ZnONPs. Values represent the mean ± standard deviation (n = 3), * - $p < 0.05$, ** - $p < 0.01$ vs. control (Student's t-test)

Concentrations of 0.01–0.5 mg/L CuNPs did not significantly alter the antioxidant activity (AA) values, with only a weak correlation observed between AA and the applied concentration (r = 0.5569). In contrast, at concentrations of 1–20 mg/L, a significant increase in AA values was recorded, with 1 mg/L CuNPs resulting in a 17.13% increase ($p < 0.05$), and 20 mg/L producing a 41.79% increase ($p < 0.01$), which corresponds to 95.3% ABTS radical scavenging inhibition. AA values increased proportionally with the CuNPs content in the cultivation medium, with a very strong positive correlation established (r = 0.9798). Treatment of the cyanobacterium with CuONPs induced different changes in AA, depending on the concentration of the nanoparticles. Low doses (0.01–0.5 mg/L) resulted in a non-significant reduction in AA, ranging from 13.65% to 14.07%. The correlation between AA values and the applied CuONPs concentration was moderate (r = 0.6686). For higher concentrations (1–20 mg/L), a significant increase in AA values was observed, with a 14.07% increase ($p < 0.05$) at 10 mg/L and a 20.79% increase ($p < 0.05$) at 20 mg/L compared to the control. These values correspond to 77.57% and 82.14% inhibition of the ABTS radical, respectively. In this range, a strong positive correlation was established between AA values and the CuONPs concentration (r = 0.9667). Thus, treatment with high concentrations of CuONPs, within the applied limits, resulted in increased antioxidant activity of the aqueous extracts.

The presence of TiO_2 and ZnO nanoparticles at concentrations of 0.01–0.5 mg/L in the cultivation medium induced a significant reduction in the antioxidant activity of the aqueous extracts, with the magnitude of the effect depending on both the nanoparticle type and the applied dose. In the case of TiO_2NPs, the reduction in antioxidant activity ranged from 24.6% ($p < 0.05$) at 0.01 mg/L to 33.0% ($p < 0.05$) at 0.5 mg/L, indicating a dose-dependent increase in the inhibitory effect. The correlation coefficient revealed a moderate negative dependence (r = –0.6791). For ZnONPs, the decrease in AA values was more pronounced and statistically significant ($p < 0.01$), with reductions of 47.4% at 0.01 mg/L and 54.8% at 0.5 mg/L, compared to the control. The correlation between AA values and ZnONPs concentration was strongly negative (r = –0.7881). These findings clearly demonstrate an adverse effect, dependent on both dose and nanoparticle type, on the antioxidant activity of *Arthrospira platensis*. A similar trend was observed for the higher concentration range (1–20 mg/L), but only for TiO_2 NPs, where AA values decreased significantly by 24.24% ($p < 0.05$) and 45.83% ($p < 0.01$) at concentrations of 5 and 20 mg/L, respectively. For this interval, a very strong negative correlation was established (r = –0.9484). Conversely, for ZnONPs, concentrations of 10–20 mg/L resulted in a significant increase ($p < 0.05$) in AA values of the aqueous extracts, by 24.26% and 35.29%, respectively, while concentrations of 1 and 5 mg/L maintained a significant decrease in AA, by 32.72% ($p < 0.05$) and 14.34% (p

< 0.05) relative to the control. The increase in AA values was sufficient to establish a very strong positive correlation between the applied concentrations and the measured AA values (r = 0.9331).

The results obtained for the ethanolic extracts revealed a distinct profile, reflecting specific alterations in the antioxidant activity of *A. platensis* biomass following nanoparticle exposure **(Figure 2**).

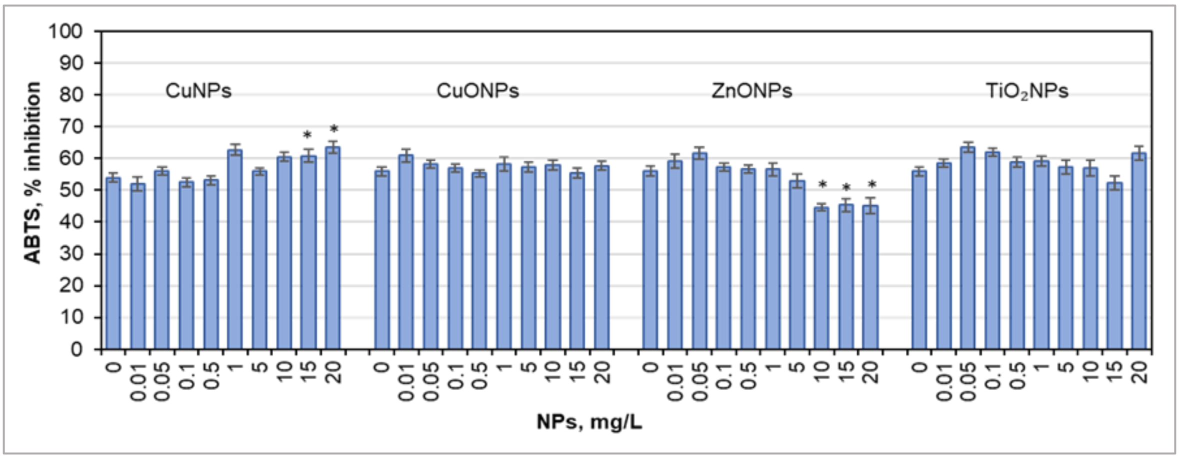

Figure 2 Antioxidant activity (% ABTS inhibition) of ethanolic extracts obtained from the biomass of *Arthrospira platensis* exposed to CuNPs, CuONPs, TiO_2NPs, and ZnONPs. Values represent the mean ± standard deviation (n = 3), * - $p < 0.05$ vs. control (Student's t-test)

At low concentrations (0.01–0.5 mg/L), the presence of CuNPs, CuONPs, TiO_2NPs, and ZnONPs induced only minor variations in antioxidant activity. At higher concentrations, the effects of nanoparticles on the ethanolic extracts from *Arthrospira platensis* biomass were more specific. Most concentrations in the range of 1–20 mg/L CuNPs resulted in a significant increase ($p < 0.05$) of 12.69% to 17.72% in antioxidant activity. A weak positive correlation was recorded between AA values and nanoparticle concentrations (r = 0.3907). In the case of ZnONPs, concentrations of 1 and 5 mg/L did not affect AA, while concentrations between 10 and 20 mg/L caused a significant decrease ($p < 0.05$) in antioxidant values by 19.13%–20.40%. A strong negative correlation was observed between the ZnONPs concentration and the AA values of the ethanolic extracts (r = –0.8668). TiO_2 nanoparticles applied at concentrations of 0.05 and 0.1 mg/L slightly increased the AA values of ethanolic extracts by 13.26% and 10.71%, respectively, although these changes were not statistically significant. Higher concentrations did not significantly alter AA values. Whereas the aqueous extracts exhibited significant variation in AA values depending on the type and concentration of nanoparticles applied, the ethanolic extracts showed changes only for two types of nanoparticles within the 1–20 mg/L concentration series.

Thus, the aqueous extracts demonstrated a much greater variability in antioxidant activity values according to nanoparticle type and concentration. CuNPs and CuONPs at high concentrations led to a significant increase in antioxidant activity, while TiO_2NPs and ZnONPs generally induced a marked decrease, with dose-dependent effects. Ethanolic extracts exhibited lower sensitivity to the presence of nanoparticles.

Some authors consider that the type of antioxidant assay is specific to the solvents used for microalgal extracts. Thus, for the ABTS assay, both aqueous and ethanolic extracts are considered effective [14]. The ABTS assay is frequently used to evaluate the antioxidant activity of microalgal extracts, allowing for the comparison of the free radical scavenging capacity of different species or experimental conditions [16]. It has been demonstrated that abiotic stress factors, including metals, can induce the accumulation of antioxidant compounds in microalgae. However, comparative data on the direct modification of antioxidant activity (as assessed by the ABTS method) in biomass cultivated in the presence of metals or nanoparticles are limited and require further investigation [2]. For example, AgNPs with sizes of 10 and 20 nm, applied to the cultivation medium, reduced the antioxidant activity values of ethanolic extracts obtained from the biomass of the microalga *Porphyridium cruentum* [12]. Several studies have reported changes in antioxidant assay values resulting from metal exposure in microalgae. For example, exposure of the microalga *Chlorella vulgaris* to various concentrations of Cu^{2+} (0–0.632 mg/L) resulted in the accumulation of compounds with increased antioxidant activity, as evaluated by the ABTS assay of methanolic extracts, with some samples exhibiting inhibition rates exceeding 90% [16]. Supplementation of the mineral medium with zinc or copper resulted in significant changes in the antioxidant activity (ABTS assay) of *Chlorella vulgaris* biomass. At moderate concentrations of Zn (0.88 mg/L) and Cu (0.158–0.316 mg/L), the antioxidant activity of the organic extract increased significantly compared to the control, reaching maximum values of 75.23% (Zn) and 78.03% (Cu), while higher concentrations of Zn (1.76 mg/L) and Cu (0.632 mg/L) induced a pronounced decrease in antioxidant activity [17].

The aqueous extract of *Arthrospira platensis* exhibited higher antioxidant activity values in the ABTS assay compared to the ethanolic extract. This result can be attributed to its high content of phycobiliproteins, carbohydrates, proteins, and peptides, which are hydrophilic compounds with a strong affinity for neutralizing the ABTS radical. The ABTS assay is particularly sensitive to these polar antioxidants, which explains the superior results for the aqueous extract relative to the ethanolic one, which contains mainly lipophilic pigments. Treatment of *Arthrospira platensis* cultures with CuNPs, CuONPs, ZnONPs, and TiO_2NPs did not result in significant changes that would affect the antioxidant assay values. For example, the protein content decreased in the case of ZnO and CuO nanoparticles, while the phycobiliprotein content increased. The chlorophyll content remained unchanged, and the carotenoid content either remained unchanged or decreased [13]. These dose-dependent increases in antioxidant activity, observed particularly at higher concentrations of CuNPs, CuONPs, and ZnONPs, may indicate the activation of adaptive physiological responses through which Arthrospira enhances or reallocates its antioxidant mechanisms to counteract nanoparticle-induced oxidative stress. Such adaptive responses involve the stimulation of pigment synthesis or the mobilization of low-molecular-weight antioxidants, mechanisms well documented in microalgae under abiotic stress where oxidative pressure activates pathways that maintain redox homeostasis. [7,13]. Thus, the results demonstrate that the tested nanoparticles significantly influence antioxidant activity, highlighting their role in modulating the antioxidant response of *Arthrospira platensis* extracts.

4. CONCLUSION

This study demonstrated that exposure of Arthrospira platensis to CuNPs, CuONPs, ZnONPs, and TiO_2NPs resulted in significant and distinct changes in antioxidant activity, depending on the nanoparticle type, concentration, and extraction solvent. Aqueous extracts showed marked, dose-dependent increases in antioxidant activity at high concentrations of CuNPs, CuONPs, and ZnONPs, whereas ethanolic extracts exhibited minor or non-significant responses. These findings indicate that metal-based nanoparticles modulate the antioxidant mechanisms of Arthrospira platensis, potentially inducing adaptive physiological responses to nanoparticle-driven oxidative stress. Given these enhanced antioxidant profiles, extracts obtained from nanoparticle-treated biomass may therefore represent high-value functional products with promising biotechnological and nutritional applications.

ACKNOWLEDGEMENTS

This research was funded by the MINISTRY OF EDUCATION AND RESEARCH OF REPUBLIC OF MOLDOVA, Research Subprogram 020101 ”InBioS”.

REFERENCES

[1] PEREIRA, L., COTAS, J., VALADO, A. Antioxidants from microalgae and their potential impact on human well-being. *Explor Drug Sci*. 2024, vol. 2, pp. 292–321. Avalaible from: https://doi.org/10.37349/eds.2024.00048.

[2] COULOMBIER, N., JAUFFRAIS, T., LEBOUVIER, N. Antioxidant Compounds from Microalgae: A Review. *Mar. Drugs.* 2021, vol. 19, pp. 1-30. Avalaible from: https://doi.org/10.3390/md19100549

[3] GHEDA, S., ABD EL-ZAHER, E.H.F., ABOU-ZEID, A.M., BEDAIR, N.A., PEREIRA, L. Potential Activity of *Arthrospira platensis* as Antioxidant, Cytotoxic and Antifungal against Some Skin Diseases: Topical Cream Application. *Mar. Drugs*. 2023, vol. 21, pp. 1-20. Avalaible from: https://doi.org/10.3390/md21030160

[4] GENTSCHEVA, G., NIKOLOVA, K., PANAYOTOVA, V., PEYCHEVA, K., MAKEDONSKI, L., SLAVOV, P., RADUSHEVA, P., PETROVA, P., YOTKOVSKA, I. Application of *Arthrospira platensis* for Medicinal Purposes and the Food Industry: A Review of the Literature. *Life.* 2023, Vol. 3(3), pp.1–20. Avalaible from: https://doi:10.3390/life13030845

[5] SAHIL, S., BODH, S., VERMA, P. *Spirulina platensis*: A comprehensive review of its nutritional value, antioxidant activity and functional food potential. *J Cell Biotech*. 2024, vol. 10(2), pp. 159–172. Avalaible from: https://doi:10.3233/JCB-240151

[6] SABAT, S., BEJ, S., SWAIN, S., BISHOYI, A.K., SAHOO, C.R., SABAT, G., PADHY, R.N. Phycochemistry and pharmacological significance of filamentous cyanobacterium *Spirulina* sp. *Bioresour. Bioprocess*. 2025, vol. 12, pp. 1–37. Avalaible from: https://doi.org/10.1186/s40643-025-00861-0

[7] KOLETTI, A., AKLIROS, D., DERVISI, I., ROUSSIS, A., ELEMETAKIS, E. Oxidative stress responses in microalgae: modern insights into an old topic. *Appl Microbiol*. 2025, vol. 5, pp. 1-37. Avalaible from: https://doi.org/10.3390/applmicrobiol5020037

[8] MARJANOVIĆ, B., BENKOVIĆ, M., JURINA, T., SOKAČ CVETNIĆ, T., VALINGER, D., GAJDOŠ KLJUSURIĆ, J., JURINJAK TUŠEK, A. Bioactive Compounds from *Spirulina* spp.—Nutritional Value, Extraction, and Application in Food Industry. *Separations.* 2024, vol. 11, pp. 1–22. Avalaible from: https://doi.org/10.3390/separations11090257

[9] PODGÓRSKA-KRYSZCZUK, I. Spirulina-An Invaluable Source of Macro- and Micronutrients with Broad Biological Activity and Application Potential. *Molecules*, 2024, vol. 29, pp. 1–24. Avalaible from: https://doi.org/10.3390/molecules29225387

[10] CURCURACI, E., MANUGUERRA, S., MESSINA, C.M., ARENA, R., RENDA, G., IOANNOU, T., AMATO, V., HELLIO, C., BARBA, F.J., SANTULLI, A. Culture Conditions Affect Antioxidant Production, Metabolism and Related Biomarkers of the Microalgae *Phaeodactylum tricornutum*. *Antioxidants*. 2022, vol. 11, pp. 1–17. Avalaible from: https://doi.org/10.3390/antiox11020411

[11] BALDISSEROTTO, C., GESSI, S., FERRARETTO, E., MERIGHI, S., ARDONDI, L., GIACÒ, P., FERRONI, L., NIGRO, M., TRAVAGLI, A., PANCALDI, S. Cultivation modes affect the morphology, biochemical composition, and antioxidant and anti-inflammatory properties of the green microalga *Neochloris oleoabundans*. *Protoplasma*. 2024, vol. 261, pp. 1185–1206. Avalaible from: https://doi.org/10.1007/s00709-024-01958-7

[12] RUDI, L., CEPOI, L., CHIRIAC, T., MISCU, V., VALUTA, A., DJUR, S. Effects of citrate-stabilized gold and silver nanoparticles on some safety parameters of *Porphyridium cruentum* biomass. *Front. Bioeng. Biotechnol*. 2023, vol. 11, pp 1–13. Avalaible from: https://doi.10.3389/fbioe.2023.1224945

[13] RUDI, L., CEPOI, L., CHIRIAC, T., DJUR, S. Interactions Between Potentially Toxic Nanoparticles (Cu, CuO, ZnO, and TiO_2) and the Cyanobacterium *Arthrospira platensis*: Biological Adaptations to Xenobiotics. *Nanomaterials.* 2025, vol. 15, pp. 1–16. Avalaible from: https://doi.org/10.3390/nano15010046

[14] MONTEIRO, M., SANTOS, R.A., IGLESIAS, P., COUTO, A., SERRA, C. R., GOUVINHAS, I., BARROS, A., OLIVA-TELES, A., ENES, P., DÍAZ-ROSALES, P. Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro- and microalgae extracts. *J Appl Phycol*. 2020, vol. 32, pp. 349–362. Avalaible from: https://doi.org/10.1007/s10811-019-01927-1

[15] RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999, vol. 26, no. 9-10, pp. 1231–1237. Avalaible from: https://doi.10.1016/s0891-5849(98)00315-3.

[16] WANG, N., PEI, H., XIANG, W., LI, T., LIN, S., WU, J., CHEN, Z., WU, H., LI, C., WU, H. Rapid Screening of Microalgae as Potential Sources of Natural Antioxidants. *Foods*. 2023, vol. 12, pp. 1–17. Avalaible from: https://doi.org/10.3390/foods12142652

[17] EL-FAYOUMY, E.A., SHANAB, S.M.M, GABALLA, H.S., TANTAWY, M.A., SHALABY, E.A. Evaluation of antioxidant and anticancer activity of crude extract and different fractions of *Chlorella vulgaris* axenic culture grown under various concentrations of copper ions. *BMC Complement Med Ther*. 2021, vol. 21, pp. 1-16. Avalaible from: https://doi.org/10.1186/s12906-020-03194-x11,76470588235